Human cells recovery from liquid nitrogen

Materials

1. Complete medium (premixed, DMEM+5%FBS) HyClone™ Dulbecco's Modified Eagles Medium
2. Flask (T25,T75)

Procedure

1. Take a vial of HEK cells from liquid nitrogen container
2. Dip it in a water bath at 37°C until the contents melt
3. Pipette out the contents with a 1 ml pipette to a T25 flask
4. Add 5 ml complete medium (DMEM+5%FBS) drop by drop to the T25 flask(slowly)
5. Disperse the cells in the flask evenly by swirling
6. Put the flask into 37°C CO_2 incubator for one day

Sub-culturing

Materials

1. 1X PBS solution
2. 0.2% trypsin solution
3. Complete medium (premixed, DMEM+5%FBS) (Gibco)
4. Flasks (T25 and T75)

Procedure

1. Place bottles of PBS and complete medium in a water bath at 37°C for at least 30 minutes
2. Take the flask from the incubator
3. Remove the medium in the flask by aspirating with a Pasteur pipette
4. Add 5 ml PBS onto the plate and swirl
5. Remove all the PBS by aspiration
6. Repeat the step 4 & 5
7. Add 0.5% trypsin (cover the area of cell growth) and swirl
8. Put the flask back in the incubator 37°C for 2 minutes
9. Add 4ml complete medium to stop the trypsin reaction
10. Calculate the split ratio and aspirate the cell content to another flask with complete medium

11. Put the flask into 37℃ CO_2 incubator for one day

Cryopreservation cultured cells

Materials

1. 1X PBS solution
2. 0.2% trypsin solution(HyClone)
3. Complete medium (premixed, DMEM+5%FBS)
4. Flasks and cryopreserve vials
5. 10% DMSO (Gibco)

Procedure

1. Follow the protocol of sub-culturing up to step 9
2. Add 9 ml cell content and 1ml 10%DMEM into a cryogenic storage vials.
3. Frequently and gently mix the cells to maintain a homogeneous cell suspension.
4. Freeze the cells in a controlled rate freezing apparatus, decreasing the temperature approximately 1°C per minute. Alternatively, place the cryovials containing the cells in an isopropanol chamber and store them at –80°C overnight.
5. Transfer the frozen cells (in -80℃ freezer) to liquid nitrogen, and store them in the gas phase above the liquid nitrogen.